PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 44.95.76948/001	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/00529	International filing date *(day/month/year)* 06.02.2003	Priority date *(day/month/year)* 06.02.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/82		
Applicant AXIS-SHIELD ASA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.09.2003	07.04.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hinchliffe, P Telephone No. +49 89 2399-8431

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/00529

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-19 as originally filed

Claims, Numbers

1-20 received on 17.02.2004 with letter of 16.02.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations

see separate sheet

ITEM V

1. The current method and kits concern a method of assaying for folate in the pABA (para aminobenzoic acid) form, with or without additional glutamate residues. The method does not require any chromatographical purification steps. The assay is therefore novel over the documents cited in the I.S.R. (Article 33(2) PCT).

2. The claims also involve an inventive step insofar as this is the first assay in which the core pABA has been used to determine total folate concentration. The closest prior art method in D2 involves chromatographical purification steps and as such the current method avoids these laborious steps in an inventive manner (Article 33(3) PCT).

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CLAIMS:

1. A method of assaying for folate in a folate containing sample, wherein at least some of said folate comprises at least one attached glutamate residue, said method comprising:

subjecting said sample to hydrolysis to release paraaminobenzoic acid, p-aminobenzoyl glutamic acid, or a salt thereof; contacting the released paraaminobenzoic acid, p-aminobenzoyl glutamic acid, salt, or a diazo derivative thereof, with a binding partner therefor; and directly or indirectly detecting the resulting binding partner:paraaminobenzoic acid, binding partner:p-aminobenzoyl glutamic acid, or salt or derivative combination wherein said method does not comprise any chromatographic separation steps.

2. A method as claimed in claim 1 wherein said sample is a blood derived sample.

3. A method as claimed in claim 1 or claim 2 wherein said binding partner is selected from an antibody, an antibody fragment, a single chain antibody, a single chain antibody fragment, an oligopeptide, an oligonucleotide and a small organic molecule.

4. A method as claimed in claim 3 wherein said small organic molecule is an aromatic tertiary amine, phenol or phenol derivative capable of forming a diazo compound with paradiazobenzoic acid (PDBA) or paradiazobenzoyl glutamate (PDBA-glu).

5. A method as claimed in any of claims 1 to 4 wherein said hydrolysis comprises treating said sample with a metal catalyst under acidic conditions.

6. A method as claimed in any of claims 1 to 5 wherein said hydrolysis comprises treating said sample with microwave radiation.

7. A method as claimed in any of claims 1 to 6 wherein said hydrolysis comprises treatment with an oxidising agent.

8. A method as claimed in claim 7 wherein the oxidising agent is hydrogen peroxide and/or potassium permanganate.

9. A method as claimed in any of claims 1 to 8 wherein said hydrolysis comprises treatment with a reducing agent.

10. A method as claimed in claim 9 wherein the reducing agent is sodium borohydride.

11. A method as claimed in any of claims 1 to 10 wherein said hydrolysis comprises oxidative photolysis.

12. A method as claimed in claim 11 wherein said oxidative photolysis is carried out in the presence of a photosensitiser.

13. A method as claimed in any of claims 1 to 12 wherein said sample is incubated in the presence of naturally occurring and/or added enzymes whereby to remove all but the terminal glutamate residue from said folate and wherein the product of the hydrolysis is PABA-glu.

14. A method as claimed in any of claims 1 to 12 wherein said sample is incubated in the presence of at least one added enzyme, whereby to remove all glutamate residues from said folate, and wherein the product of

the hydrolysis is PABA.

15. A method as claimed in any of claims 1 to 14 wherein said binding partner:paraaminobenzoic acid, binding partner:p-aminobenzoyl glutamic acid, or salt or derivative combination is detected directly by absorbence or fluorescence.

16. A method as claimed in any of claims 1 to 14 wherein said binding partner:paraaminobenzoic acid, binding partner:p-aminobenzoyl glutamic acid, or salt or derivative combination is detected indirectly by means of a secondary binding partner.

17. A kit for use in the performance of the assay of the invention, said kit comprising:

i) a folate hydrolysis reagent; and

ii) a PABA, PABA-glu, PDBA or PDBA-glu binding partner;

18. A kit as claimed in claim 17 additionally comprising an enzyme or enzyme cocktail.

19. A kit as claimed in claim 17 or claim 18 additionally comprising a PABA to PDBA or PABA-glu to PDBA-glu converting reagent.

20. A kit as claimed in any of claims 17 to 19 additionally comprising a secondary binding partner.